Aire, Master of Many Trades

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In thymic epithelial cells, the protein Aire (autoimmune regulator) induces the ectopic expression of hundreds of peripheral tissue antigens, thus enlarging the repertoire of antigens available for the induction of central T cell tolerance. By analyzing Aire's interacting partners, Abramson et al. (2010) shed new light on this unorthodox form of gene expression.

T cells, B cells, and natural killer T (NKT) cells recognize antigens via surface receptors, and because these receptors are generated by genetic recombination in a random fashion, they encompass specificities for both foreign and self-antigens. The latter pose a danger and need to be weeded out during immune cell maturation (central tolerance) or controlled by various specialized subsets of regulatory cells in peripheral tissues (peripheral tolerance). The process of ridding the nascent repertoire of self-reactive T cells in the thymus is termed negative selection. Negative selection is based on the scanning of self-antigens presented by various antigen-presenting cells in the thymus, notably in the medullary compartment. The spectrum of proteins against which central tolerance is induced is therefore dictated by the array of self-antigens available to thymic antigen-presenting cells, which include dendritic cells and thymic medullary epithelial cells (MECs) (Klein et al., 2009). Although it was once assumed that the tissue-specific antigens in the thymus

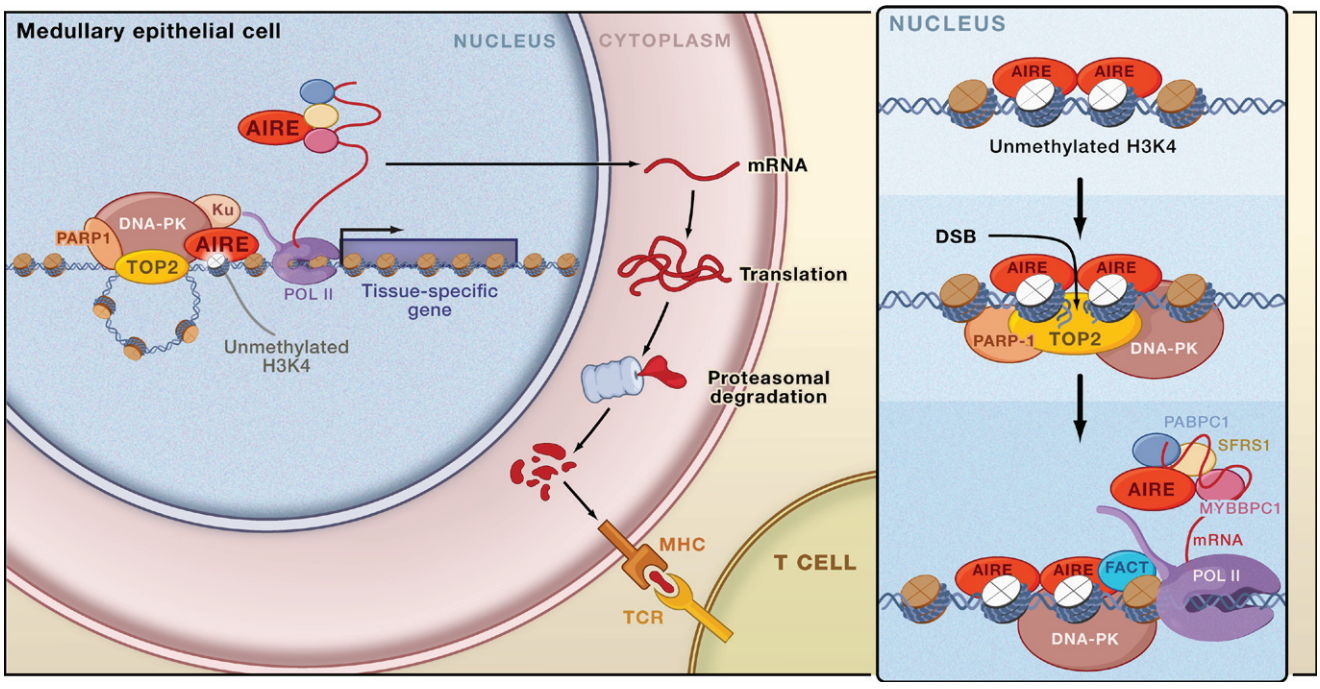

Figure 1. Aire's Multiple Partners
Aire (autoimmune regulator) is involved in many different steps of transcription in order to foster ectopic gene expression in medullary epithelial cells (MECs). The temporal order depicted in this model (right panel) remains speculative. Aire binds via its PHD1 domain to unmethylated lysine 4 of histone 3. Lack of histone H3 lysine 4 methylation characterizes the regulatory regions of silent genes, such as those that encode peripheral tissue antigens in MECs. Aire also recruits a group of proteins that promote transcriptional elongation by inducing single- and double-strand breaks and by removing and reassembling histones around RNA polymerase II (Pol II). Aire also enhances the processing of pre-mRNA to mature mRNA. Proteins translated from mature mRNA will be processed and presented via major histocompatibility complex class-I and -II to immature thymocytes in the thymic medulla. High avidity interactions between these peptide/MHC complexes and the T cell receptors of self-reactive T cells will eventually result in their apoptosis (negative selection). DNA-PK, DNA-dependent protein kinase; DSB, double-strand break; FACT, facilitates chromatin transcription; H3K4, histone 3 lysine 4; Ku, Ku80; MYBBP1A, MYB-binding protein 1A; Pol II, RNA polymerase II; PABPC1, poly(A)-binding protein C1; PARP-1, poly (ADP-ribose) polymerase 1; SFRS1, splicing factor arginine/serine-rich 1; TOP2, topoisomerase 2a.

only represented a minor fraction of the total repertoire of self-antigens in the body, it is now known that the diversity of thymic self-antigens is considerably enriched by the ectopic, or promiscuous, expression of numerous peripheral tissue antigens by MECs (Kyewski and Klein, 2006). Yet, elucidating the molecular basis for promiscuous gene expression by MECs has proven daunting. The study in this issue by Abramson et al. (2010) represents a remarkable stride forward in this pursuit.

The focal point for this work is Aire (autoimmune regulator), a protein highly expressed in MECs. Prior work has demonstrated that mutations in human AIRE result in a rare multiorgan autoimmune disease, *a*utoimmune *p*olyendocrinopathy-candidiasis-ectodermal *d*ystrophy (APECED). Mice lacking Aire develop a similar syndrome and MECs from these mice exhibit a decrease in promiscuous gene expression (Anderson et al., 2002). Although the cellular regulation of Aire and its impact on negative selection are now well-characterized, identifying its molecular modes of action has proven more challenging. Whatever these exact mechanism(s) are, all models for Aire function need to accommodate the following observations: Aire targets an unusual diversity of genes that are dispersed throughout the whole genome; genes dependent on Aire for activation are highly enriched in tissue-specific genes, and they are preferentially arranged in clusters in the genome; the degree of Aire dependency varies widely between different genes, and there is a clear dosage effect between Aire protein levels and the degree of transcriptional activation; Aire is necessary but not sufficient for transcription of its target genes at the single-cell level; and, lastly, expression of Aire-dependent genes in single MECs appears stochastic.

Accordingly, a plethora of proposals has been offered for Aire's potential mechanisms of action, ranging from direct DNA binding, assembly into a larger DNA-binding complex, induction of RNA elongation by recruiting the kinase pTEFb (positive transcription elongation factor b), direct binding to histone H3 (when it is not methylated at lysine 4), or recruitment of genes to the nuclear matrix (reviewed in Peterson et al., 2008). The study by Abramson et al. provides a framework to reassess and assemble these scattered pieces of the puzzle, confirming some, adding new ones, and refuting others.

The unbiased approach taken by the Mathis group is a tour de force. Using a combination of mass-spectrometry screening, protein immunoprecipitation, and RNA interference, they identify a large set of Aire-interacting partners in cell lines. These interacting proteins can be divided into four separate groups based on their known functional roles: chromatin structure and DNA-damage response, gene transcription, RNA processing, and nuclear transport. Indeed, many of these proteins have been previously shown to interact with each other and to form large protein complexes. Aire has also been shown to partake in large protein complexes (Halonen et al., 2004). It should be emphasized that this experimental approach might preferentially select for stable interacting partners, whereas transient or weak interactions (for instance, with kinases or acetyltransferases) might be missed. Based on this comprehensive analysis the authors conclude that Aire is involved in three discernible processes (Figure 1).

The first process involves a complex that contains (in addition to Aire) DNA-PK (DNA-dependent protein kinase), TOP2 (topoisomerase 2), PARP-1 (poly ADP ribose polymerase 1), FACT (facilitates chromatin transcription), and Ku (a protein that binds the ends of DNA double-strand breaks). The complex promotes transcriptional elongation by the induction and religation of single- and double-strand DNA breaks (Nitiss, 2009). This maneuver is necessary to resolve supercoiling of DNA that is induced during the unwinding of chromatin for access by the transcription machinery. In addition, this protein complex might participate in removing and reassembling histones around elongating RNA polymerase II. This mode of action is further supported by Aire's ability to augment TOP2-induced double-strand breaks, hence acting in a similar manner to the well-known TOP2 inhibitor, etoposide. An essential participation of DNA-PK in the action of Aire (Liiv et al., 2008) is convincingly illustrated by the reduction in the expression of Aire-dependent genes in MECs of mice deficient in the *DNA-PK* gene. Aire-dependent gene expression is, however, only partially affected in DNA-PK-deficient mice, and in contrast to Aire knockout mice, DNA-PK-deficient mice do not show overt signs of autoimmunity.

The second complex controls pre-mRNA processing, which presumably occurs in nuclear speckles. This complex consists of Aire and at least six additional factors, the splicing factors SFRS and SFRS3, the putative DEAD box helicases DDX5 and DDX17, MYB-binding protein 1a, and poly(A) binding protein C. An essential function of Aire in mRNA processing is illustrated by the selective upregulation by Aire of spliced mRNA but not of pre-mRNA. This stands in contrast to classical transcription factors like Foxp3. The participation of Aire in both processes is thought to enhance transcription of weakly expressed genes, such as peripheral tissue antigens in MECs.

A third role for Aire, which is presumably the first step in this cascade, is the recognition of unmethylated histone H3 lysine 4 by Aire's PHD1 domain. This interaction is thought to confer upon Aire a degree of specificity in gene targeting (Org et al., 2008).

Although certain facets of this overall scenario have been suggested previously, the structural and functional analyses of Abramson et al. integrate them and put them on a firm footing. Importantly, the study not only relies on in vitro transfection experiments for its conclusions but also adds further support from analyses of MECs ex vivo.

Given the complexity of Aire function, it is not surprising that the study leaves many issues to be resolved, some of which have been raised by this new data. Can we necessarily assume that all these factors also interact with Aire in MECs? How might this model explain the heterogeneous and stochastic expression pattern of Aire-dependent genes in MECs at the single-cell level? Is binding of Aire to unmethylated H3K4 the decisive clue to explain the selection of Aire target genes in MECs or, for that matter, other cell types, such as peripheral lymphoid stromal cells? Are gene regions surrounding Aire-dependent genes selectively depleted of histone H3K4 methylation?

How are peripheral tissue antigens that are Aire independent regulated, given that they are often found in the genome among Aire-dependent genes? Do they employ a similar strategy that includes a role for the DNA-PK complex but instead replace Aire by another master regulator? Lastly, are the genes targeted by Aire in MECs, as compared to those in other cell types (Guerau-de-Arellano et al., 2008), specifically selected to represent the "peripheral self," for example due to a unique complement of modifying factors or chromatin configuration? Regardless of how the answers turn out, Abramson et al. provide us with a persuasive explanation for how Aire, as a single factor, "wakes up" regions of inactive chromatin leading to low-level expression of hundreds of genes in a terminally differentiated cell type devoid of the transcription factors and chromatin configurations that regulate transcription of the respective genes in tissue cells. Remarkably, Aire not only promotes promiscuous gene expression, it also engages in promiscuous partnerships.

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Plant Chromatin Feels the Heat

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Temperature is a key environmental signal regulating plant development, but the mechanisms by which plants sense small changes in ambient temperature have remained elusive. Kumar and Wigge (2010) now reveal that eviction of the histone variant H2A.Z from nucleosomes performs a central role in plant thermosensory perception.

Accurate monitoring of ambient temperature is fundamental to the survival of living organisms. Animals display marked temperature preferences and physically move to optimal thermal surroundings (Hamada et al., 2008). In contrast, plants must adapt their developmental program in response to environmental signals. Temperature can dramatically modify the growth and reproductive strategy of plants, yet little is known of the molecular mechanisms underlying such developmental plasticity. In this issue, Kumar and Wigge (2010) provide a major advance in our understanding of how plants detect changes in ambient temperature.

The majority of research to date has focused on plant adaptation to temperature extremes, such as cold and heat stress (reviewed in Penfield, 2008). In freezing-sensitive species, a prolonged period of cold can initiate signaling cascades and metabolic adaptations that enhance plant survival at subzero temperatures. Exposure to stressful high temperatures can initiate the synthesis of heat-shock proteins (HSPs) that confer some protection against protein denaturation and maintain cellular function. Small fluctuations in ambient growth temperature can, however, also have dramatic effects on plant development. When grown at cooler temperatures, many plants display a compact architecture and delay flowering. In contrast, elevated temperatures result in a graded increase in the elongation of plant axes and acceleration of the transition to reproductive development through the floral integrator FLOWERING TIME (FT) (Balasubramanian et al., 2006).

In an exciting new advance, Kumar and Wigge (2010) reveal that chromatin has a key role in the detection of changes in ambient temperature (Figure 1). The authors exploit the graded thermal response of *HSP70* expression in a forward genetic screen to isolate mutants displaying aberrant thermosensitivity. This elegant strategy results in the isolation of multiple alleles of *arp6*. ARP6